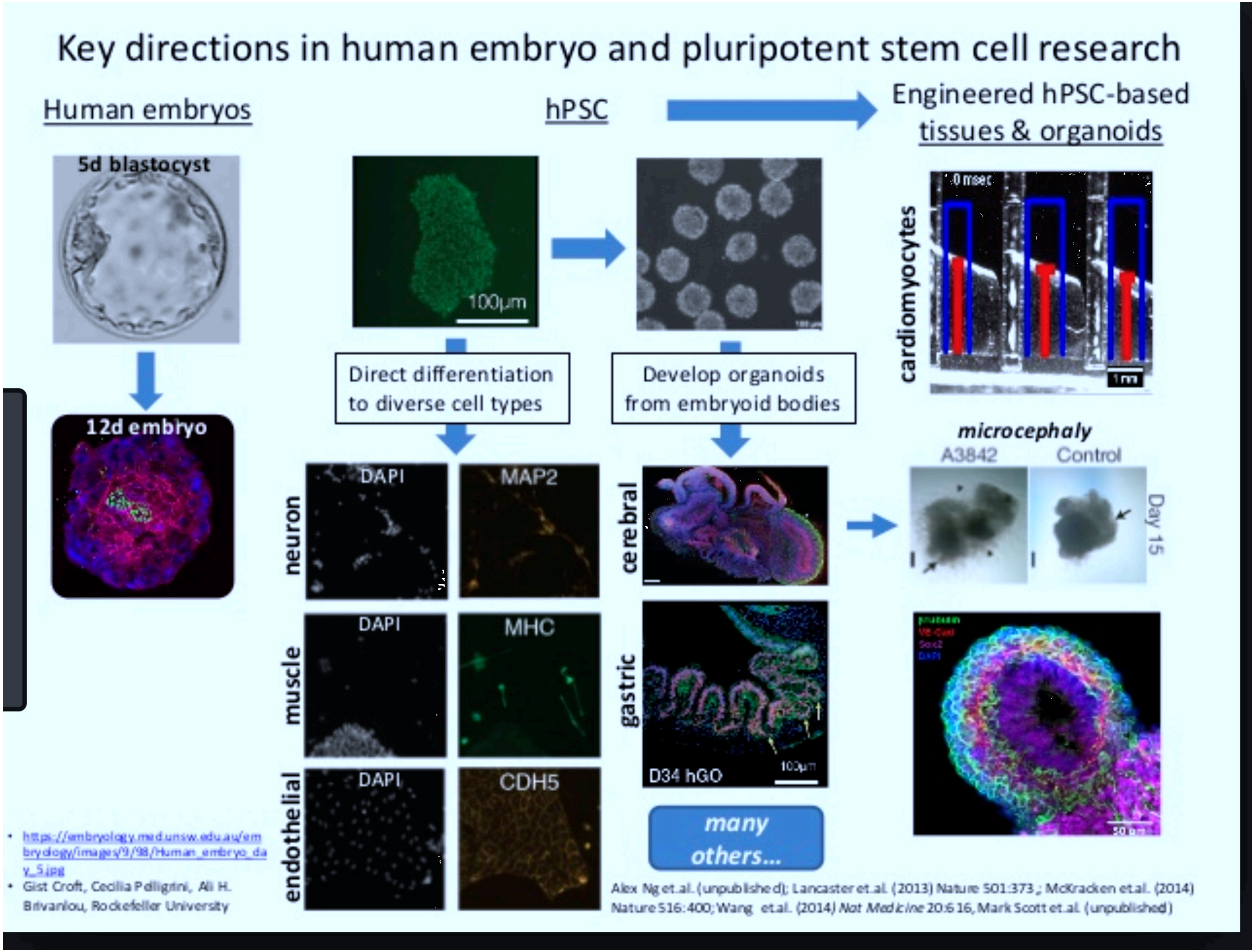
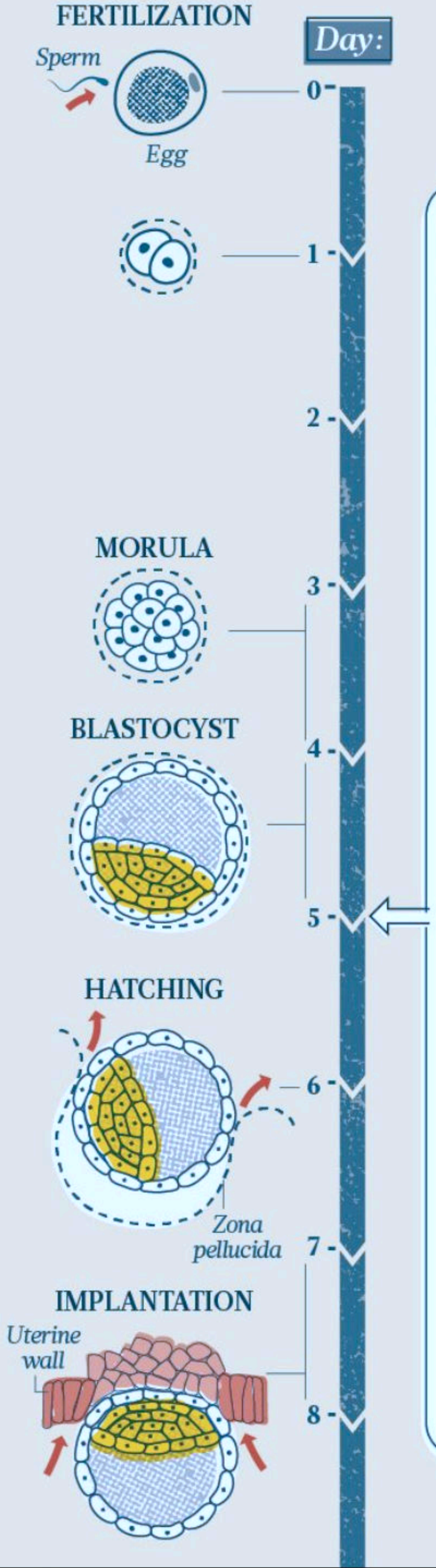
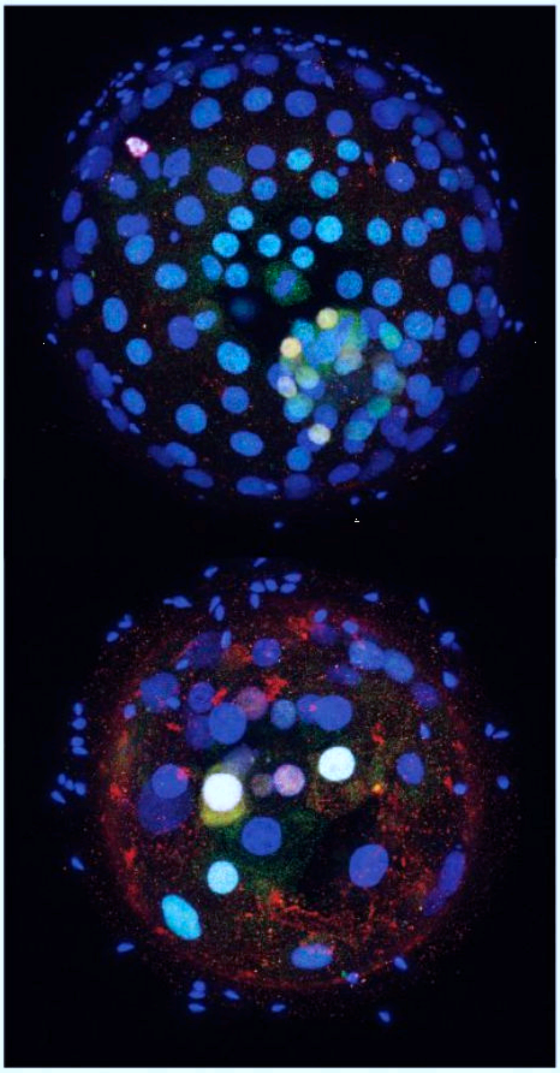

Key directions in human embryo and pluripotent stem cell research
Human embryos
hPSC
Engineered hPSC-based tissues & organoids
5d blastocyst
12d embryo
100µm
Direct differentiation to diverse cell types
Develop organoids from embryoid bodies
cardiomyocytes
0 msec
1 mm
microcephaly
A3842
Control
Day 15
neuron
DAPI
MAP2
muscle
DAPI
MHC
endothelial
DAPI
CDH5
cerebral
gastric
D34 hGO
100µm
many others...
https://embryology.med.unsw.edu.au/embryology/images/9/98/Human_embryo_day_5.jpg
Gist Croft, Cecilia Pellegrini, Ali H. Brivanlou, Rockefeller University
Alex Ng et.al. (unpublished); Lancaster et.al. (2013) Nature 501:373 ,; McKracken et.al. (2014) Nature 516:400; Wang et.al. (2014) Nat Medicine 20:616, Mark Scott et.al. (unpublished)

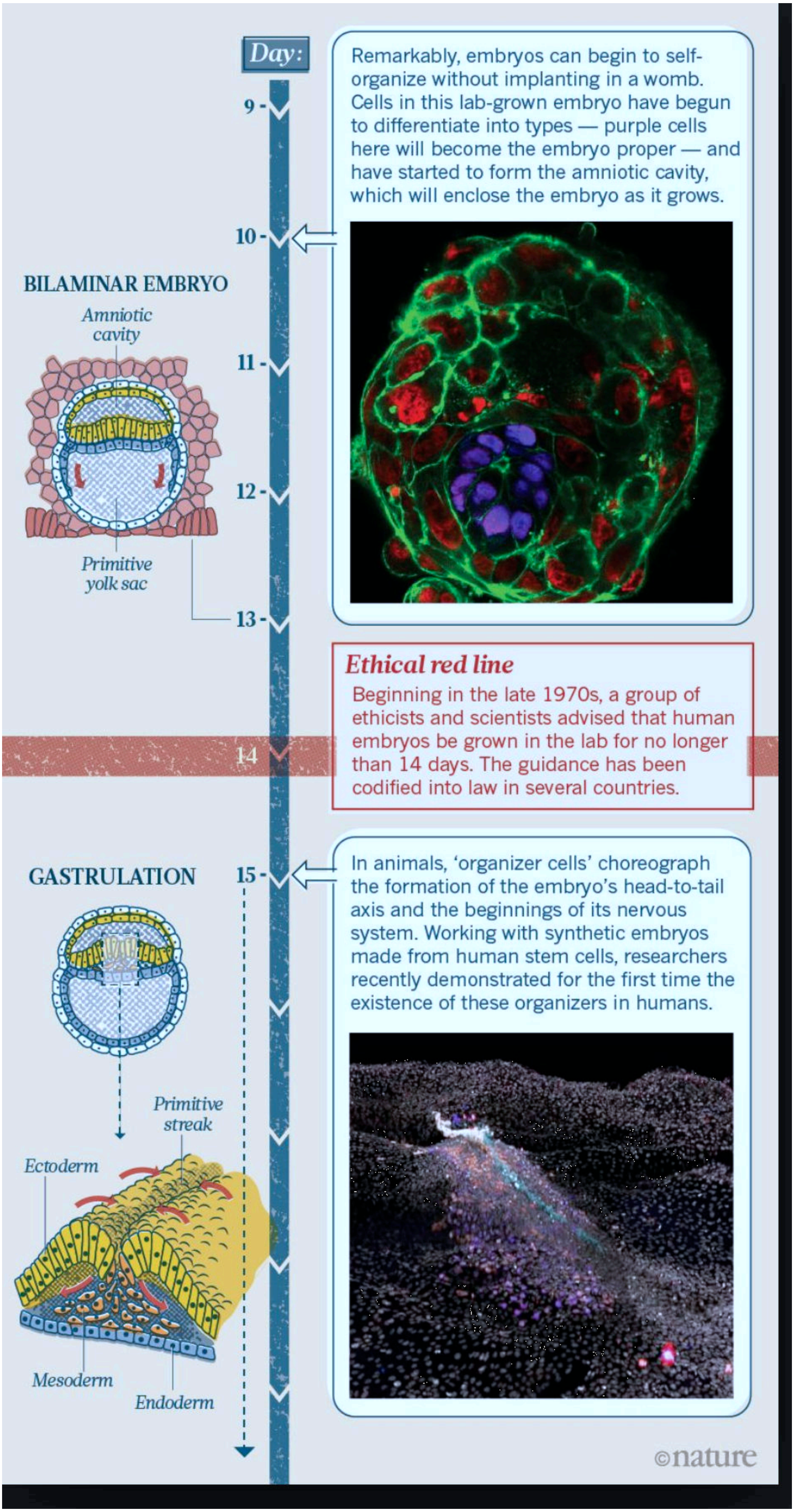
Baby steps

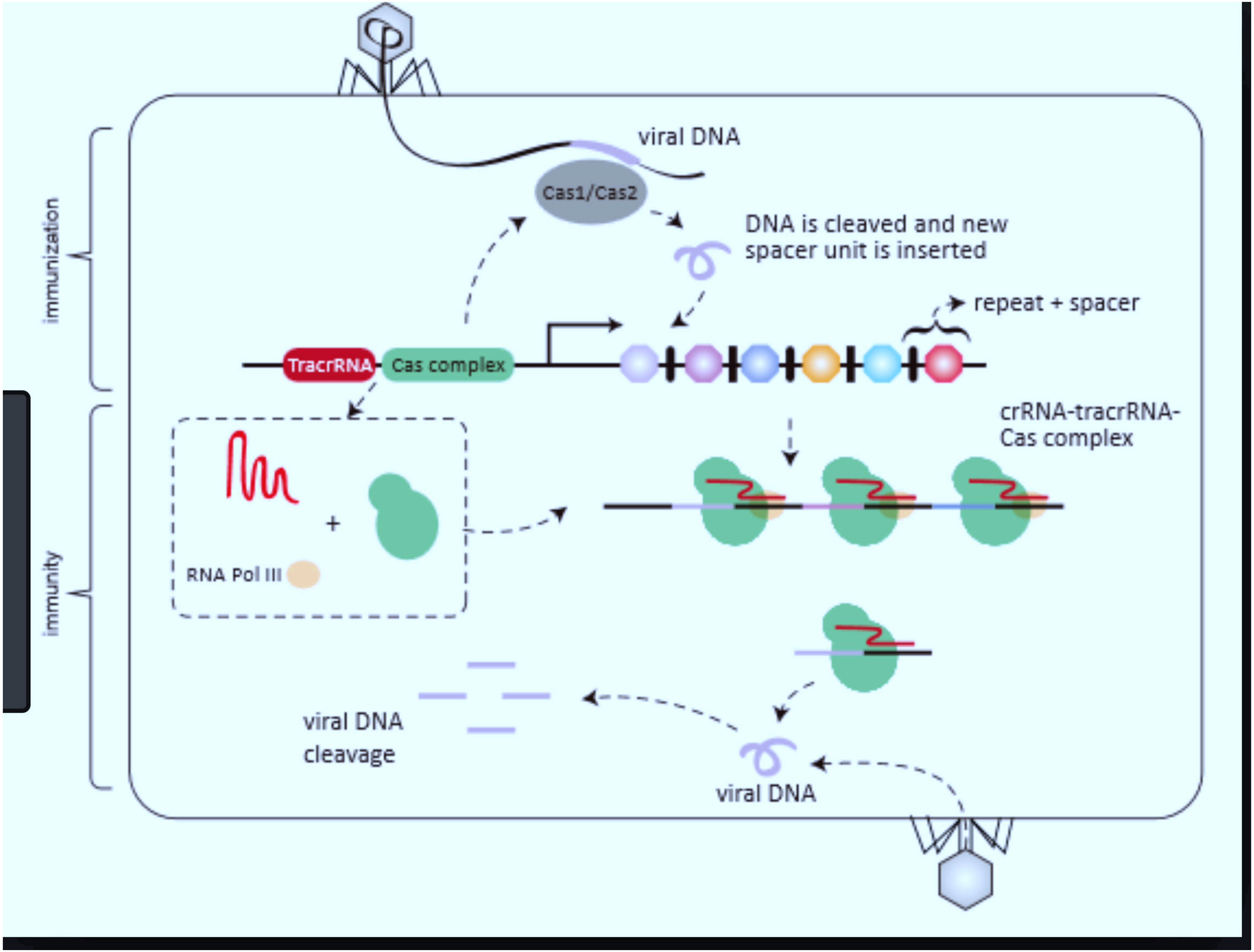
For decades, researchers could only guess at the early stages of human development, using animal studies and rare tissue samples as a guide. New methods for nurturing whole human embryos in the lab — and building embryo-like constructs from human stem cells — are starting to crack open the black box.



Using the gene-editing technique CRISPR, researchers blocked a key protein in early development called OCT4 and watched human embryos fail to grow into 200-cell blastocysts. Mouse embryos lacking OCT4 faltered at a later stage, hinting at differences between the two species even very early in development.




Day:
9
10
11
12
13
14
15
BILAMINAR EMBRYO
Amniotic cavity
Primitive yolk sac
Remarkably, embryos can begin to self-organize without implanting in a womb. Cells in this lab-grown embryo have begun to differentiate into types — purple cells here will become the embryo proper — and have started to form the amniotic cavity, which will enclose the embryo as it grows.
Ethical red line
Beginning in the late 1970s, a group of ethicists and scientists advised that human embryos be grown in the lab for no longer than 14 days. The guidance has been codified into law in several countries.
GASTRULATION
Primitive streak
Ectoderm
Mesoderm
Endoderm
In animals, 'organizer cells' choreograph the formation of the embryo's head-to-tail axis and the beginnings of its nervous system. Working with synthetic embryos made from human stem cells, researchers recently demonstrated for the first time the existence of these organizers in humans.
©nature

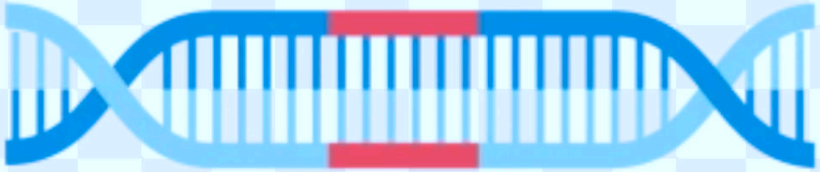


viral DNA
Cas1/Cas2
DNA is cleaved and new
spacer unit is inserted
repeat + spacer
immunization
TracrRNA
Cas complex
crRNA-tracrRNA-
Cas complex
+
RNA Pol III
immunity
viral DNA
cleavage
viral DNA

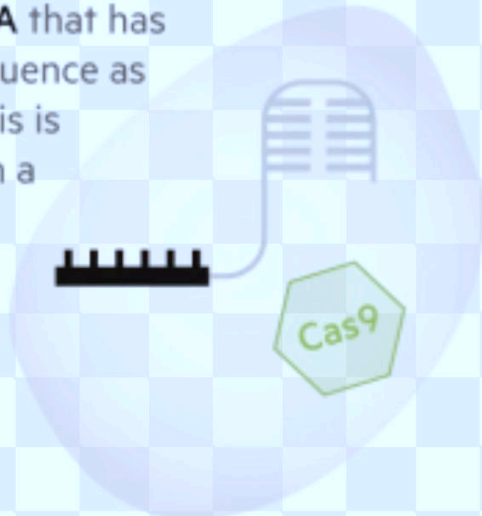
How DNA defects can be edited out

The Crispr-Cas9 technique can fight sickness at its source

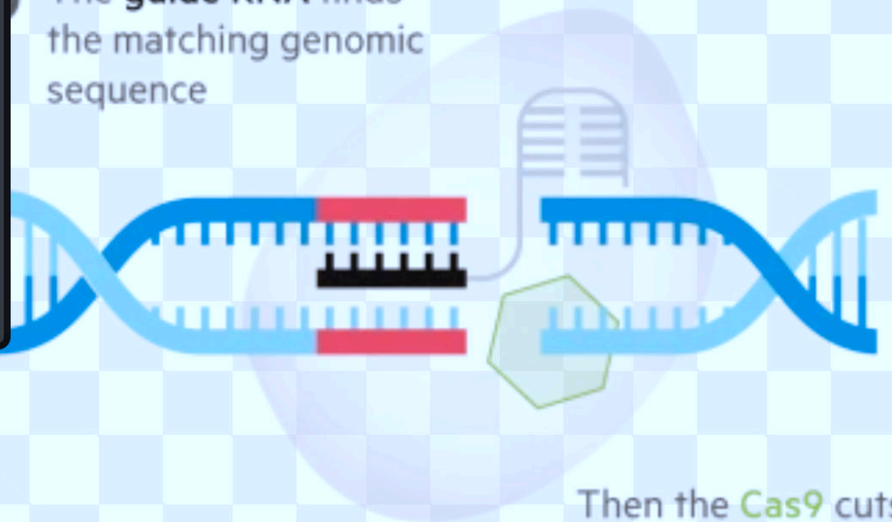
1. Scientists identify a **defective DNA strand** to be cut out and modified



2. They create **guide RNA** that has the same genomic sequence as the defective DNA. This is combined in a cell with a protein called Cas9 which acts like sissors to cut the defective DNA



The **guide RNA** finds the matching genomic sequence



Then the Cas9 cuts the strand making a break in the DNA helix

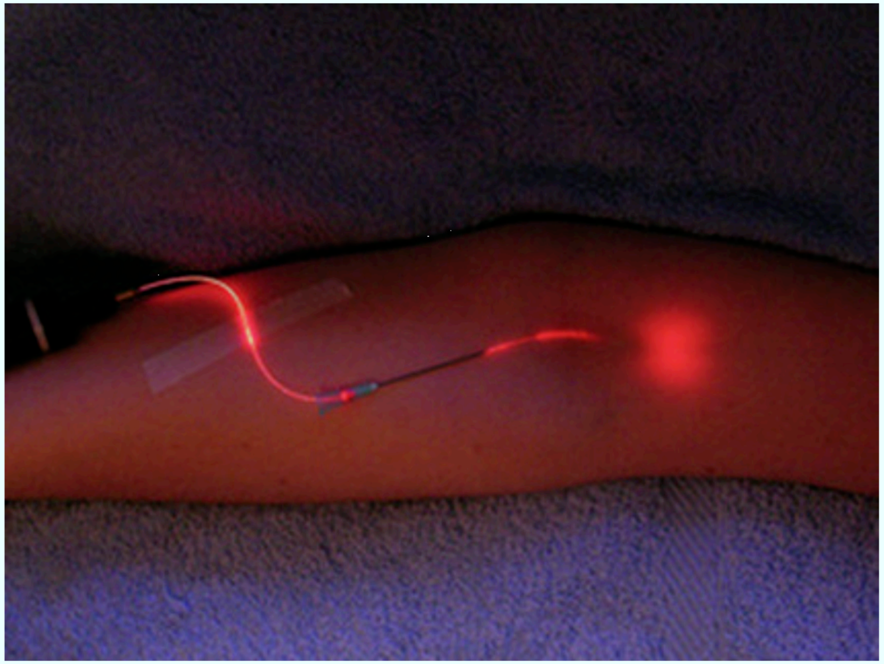
4. Cells are able to detect and repair broken DNA. A healthy strand of DNA is inserted at the cut site and enzymes repair it

Cell repair systems can use a piece of complimentary DNA, called a template. Scientists can add beneficial changes to the template, such as correcting a disease-causing mutation

Ultraviolet Blood Irradiation

This is not an endorsement for this medical treatment; see your own physician for any medical problems.

UV Blood Irradiation - educational purposes only, not medical advice.



UBI--Irradiating Blood For Infections

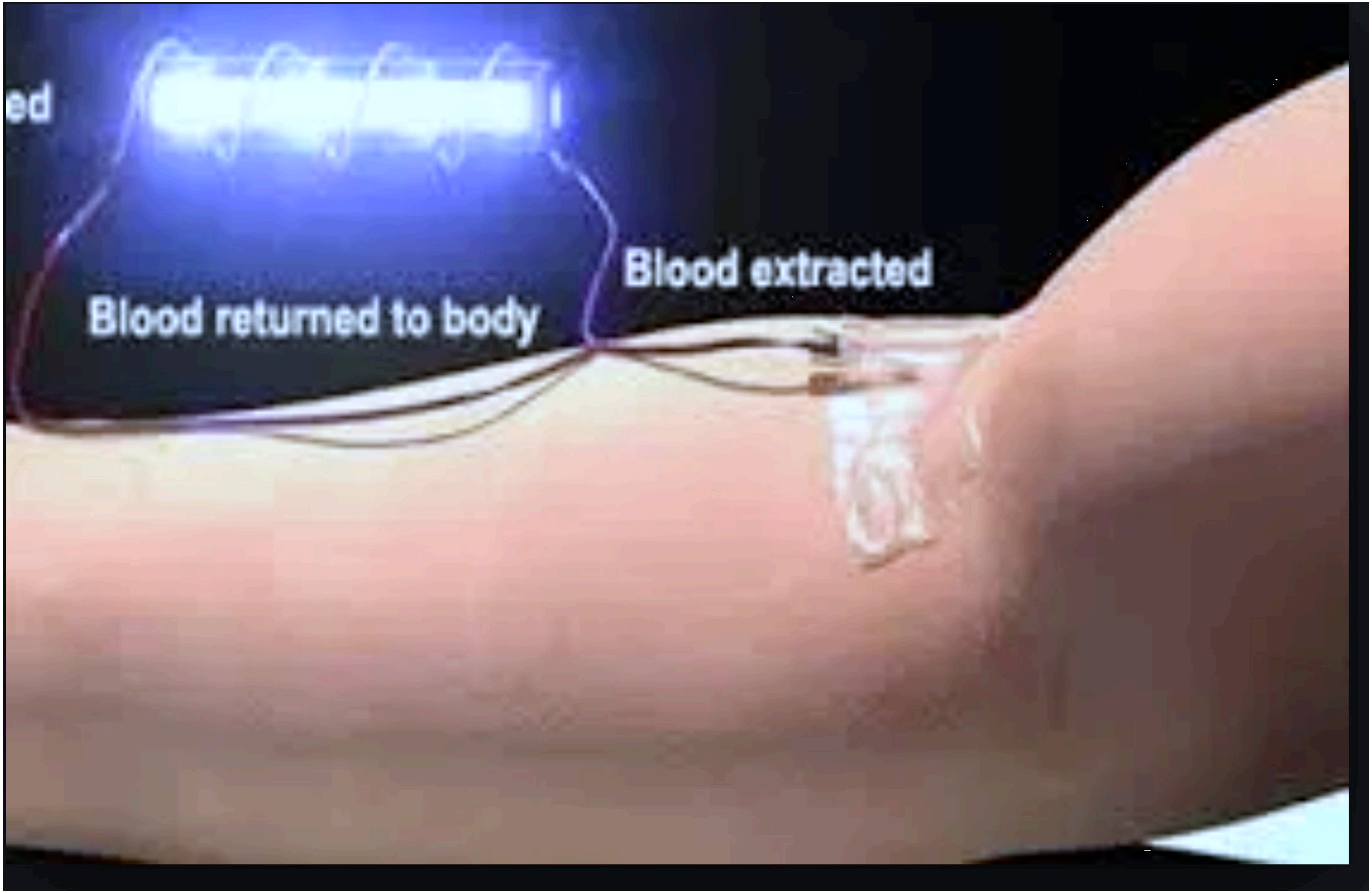
Application of Ultraviolet Blood Irradiation for Treatment of HIV and Other Blood borne Viruses

by Dr. Carl SchleicherFoundation for Blood Irradiation
Note:Dr. Schleicher died in 1999

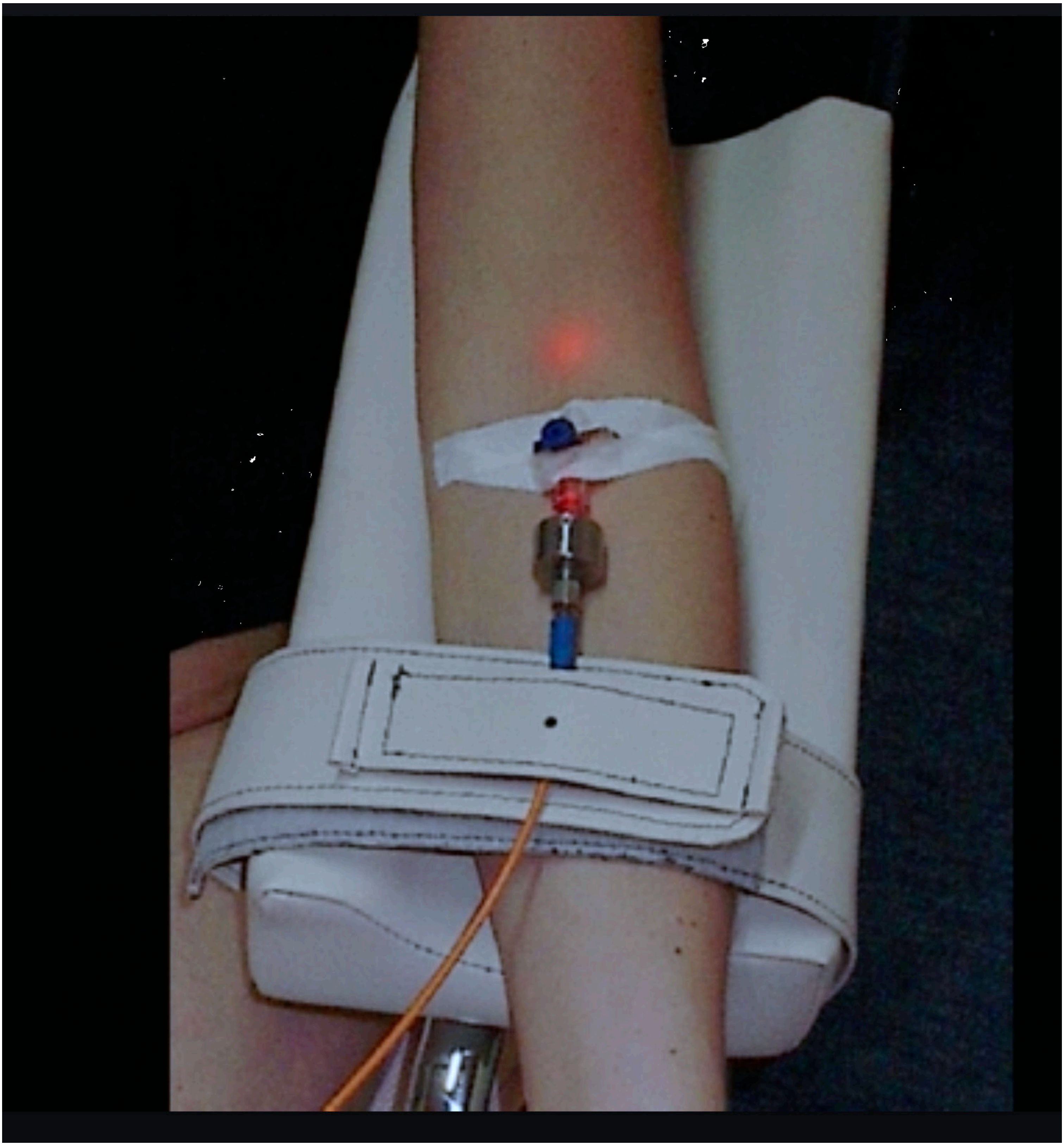
Abstract

This paper describes an innovative method of inactivating blood-borne viruses using ultraviolet blood irradiation called UBI therapy. This process has shown impressive clinical results in treating hepatitis, HIV, and other currently untreatable viruses. The background, theory, and method of using UBI therapy is presented in this paper. This method offers a potential break-through in the treatment of viral diseases and bacteria, and is nontoxic, uses no drugs, and even has FDA certification, and thus is available now for use.

Ultraviolet blood irradiation first evolved in the early 1930s as a means to treat persons afflicted with the poliovirus which was causing considerable anguish and fear similar to the advent of the HIV in the 1980s and continuing. Then in the 1950s the Salk vaccine wiped out polio in the U.S. and, as a result of this fact and other reasons, this process fell in disuse until recent years. This process has now been resurrected by the Foundation for Blood Irradiation (FFBI) which had been originally founded in the 1940s by the developers of this process, most of whom are now deceased, who left this to the next generation of researchers to continue. Much credit for the early development of this technology goes

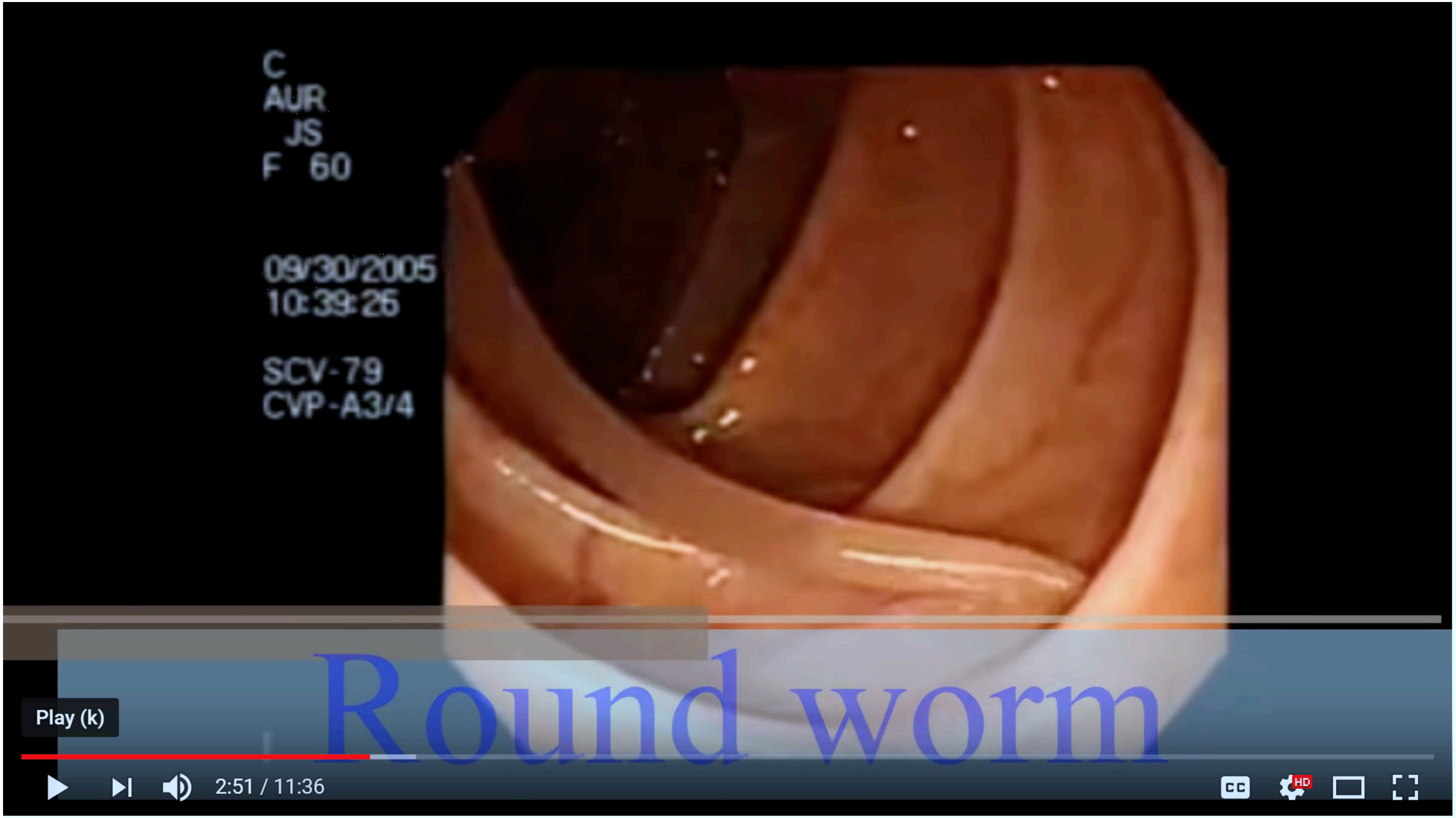

ed
Blood extracted
Blood returned to body


Champion
4000



Parasites during colonoscopy


Whipworm
0:37 / 11:36

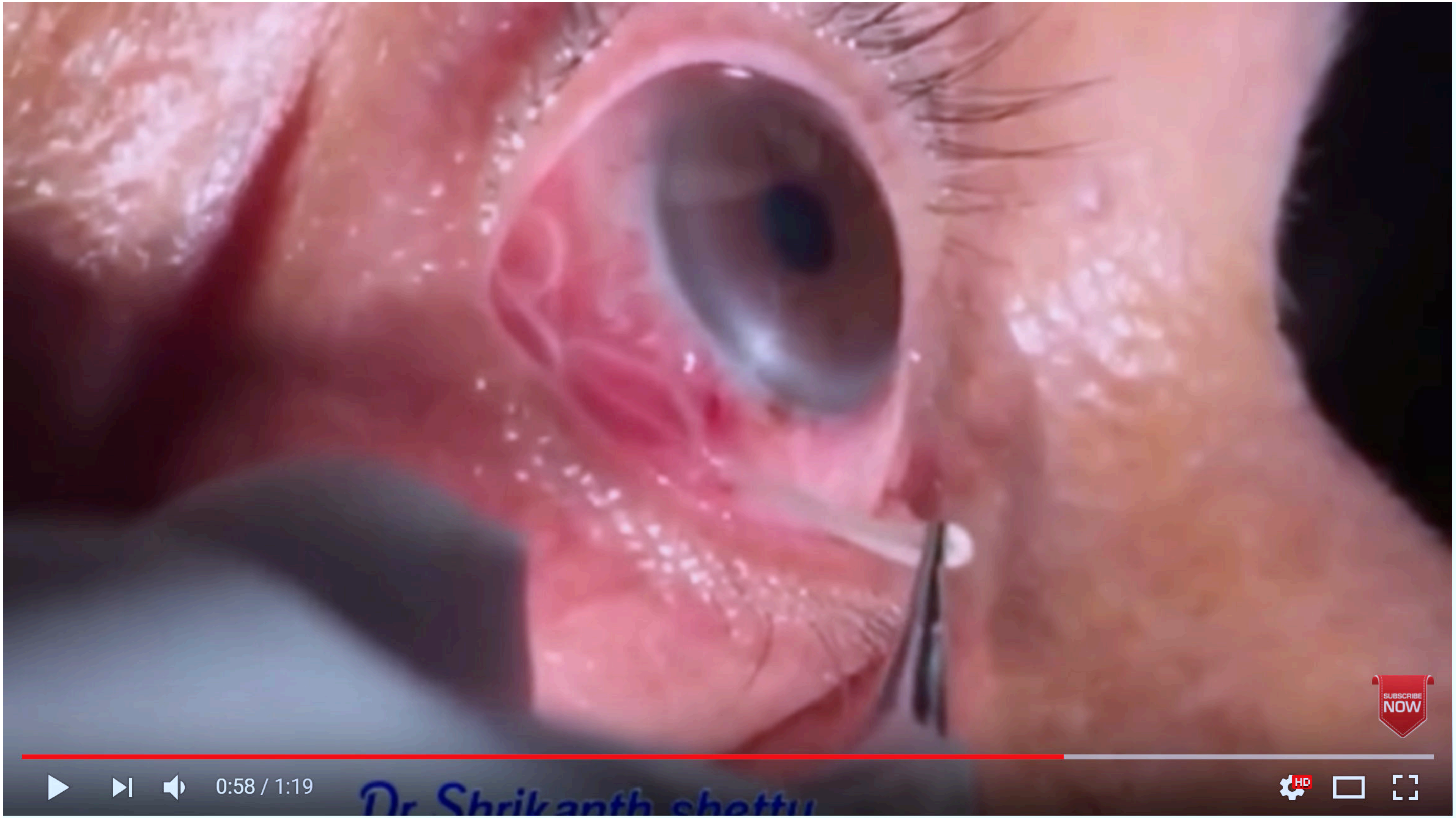

C
AUR
JS
F 60
09/30/2005
10:39:26
SCV-79
CVP-A3/4
Round worm
Play (k)
2:51 / 11:36

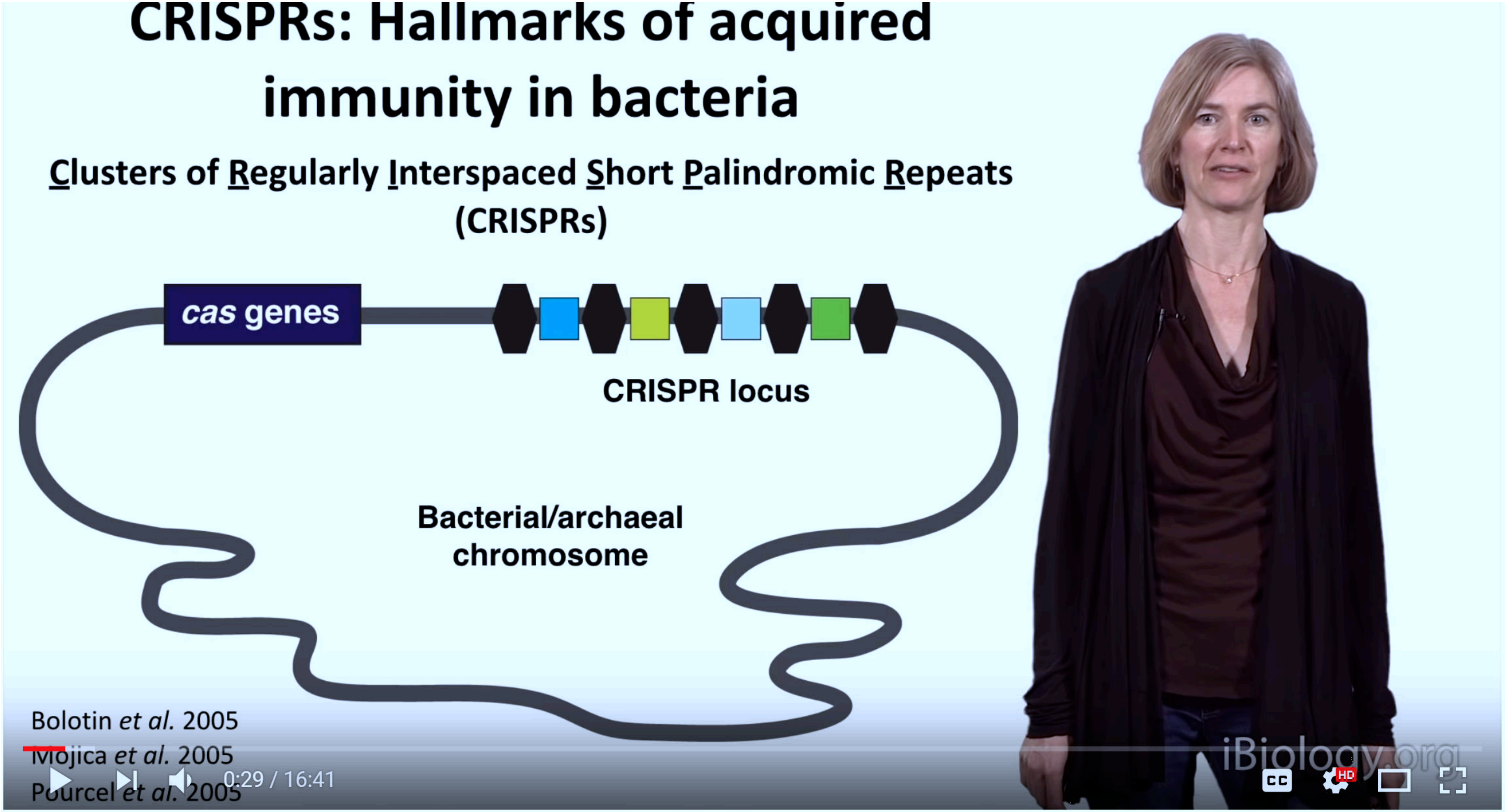

Ascaris Lumbricoides
Play (k)
3:38 / 11:36

Removing Intestinal Worms & Parasites from Bodybuilders Colon in NY RE | Intestinal Worms

VRIL Parasite FOUND!?

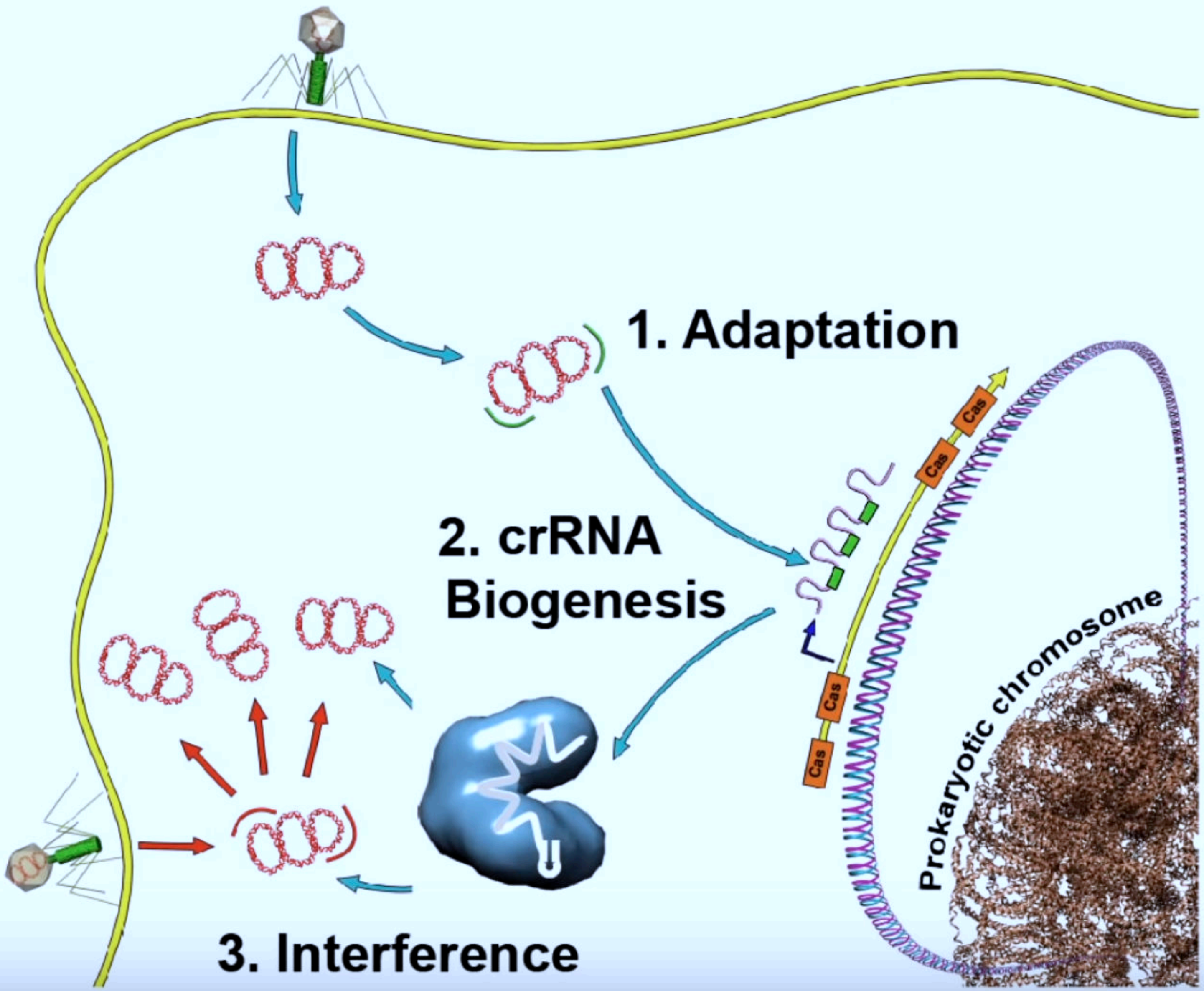

1:51 / 2:42
CC


SUBSCRIBE
NOW
0:58 / 1:19
HD

Jennifer Doudna (UC Berkeley / HHMI): Genome Engineering with CRISPR-Cas9

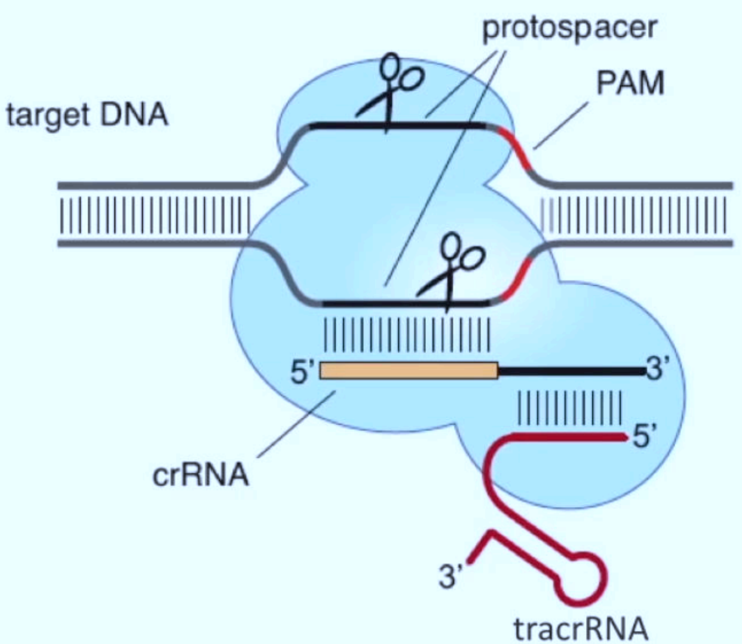
Three steps to acquire immunity in bacteria



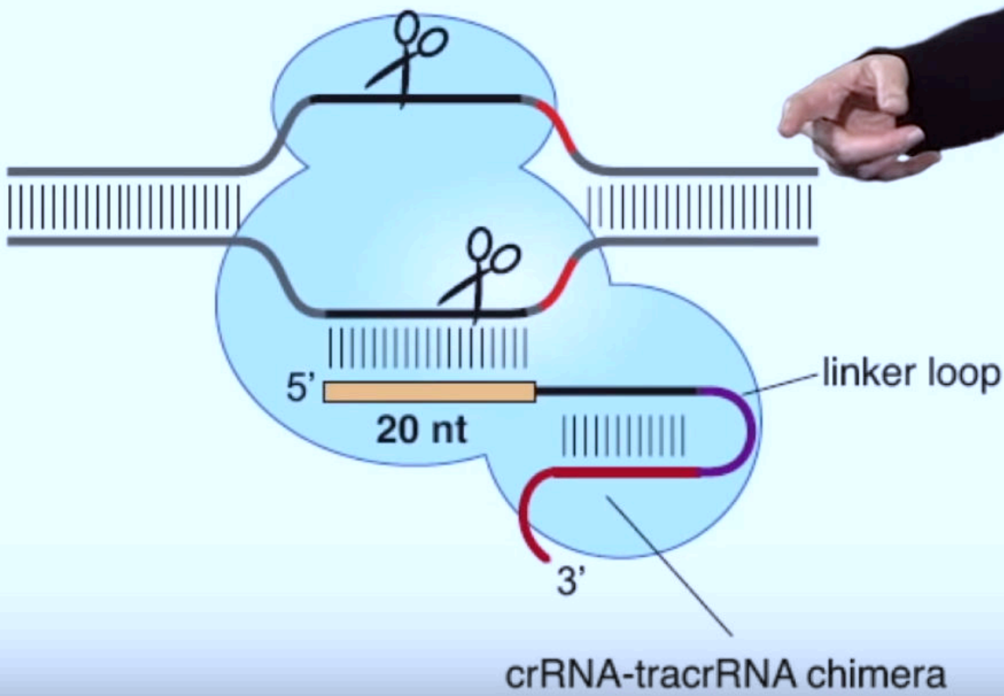
Reviews: Horvath & Barrangou (2010) *Science*; Wiedenheft et al. (2012) *Nature*

Programming Cas9 with single-guide RNAs (sgRNAs)

Cas9 programmed by crRNA:tracrRNA duplex

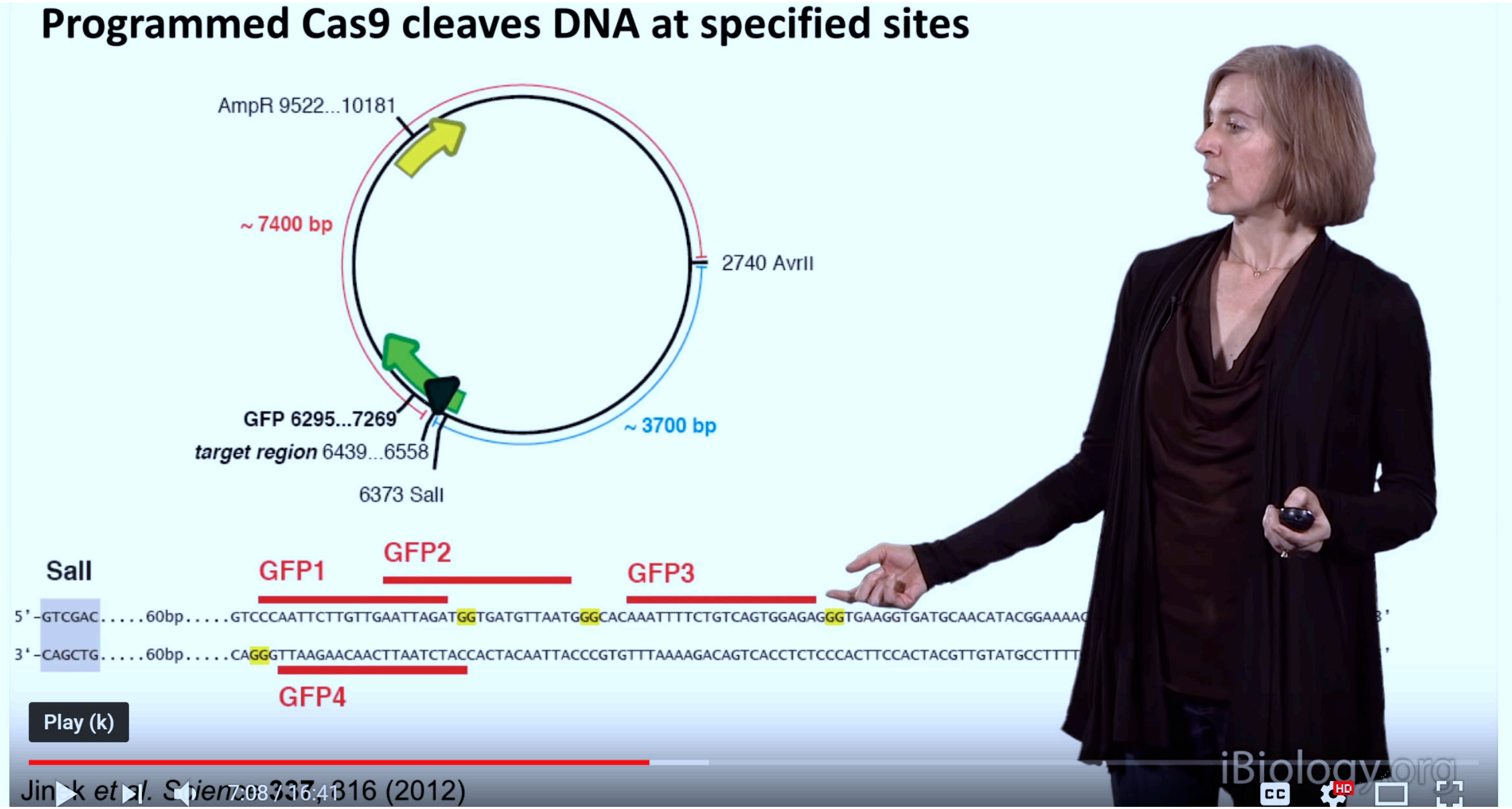


Cas9 programmed by single chimeric RNA



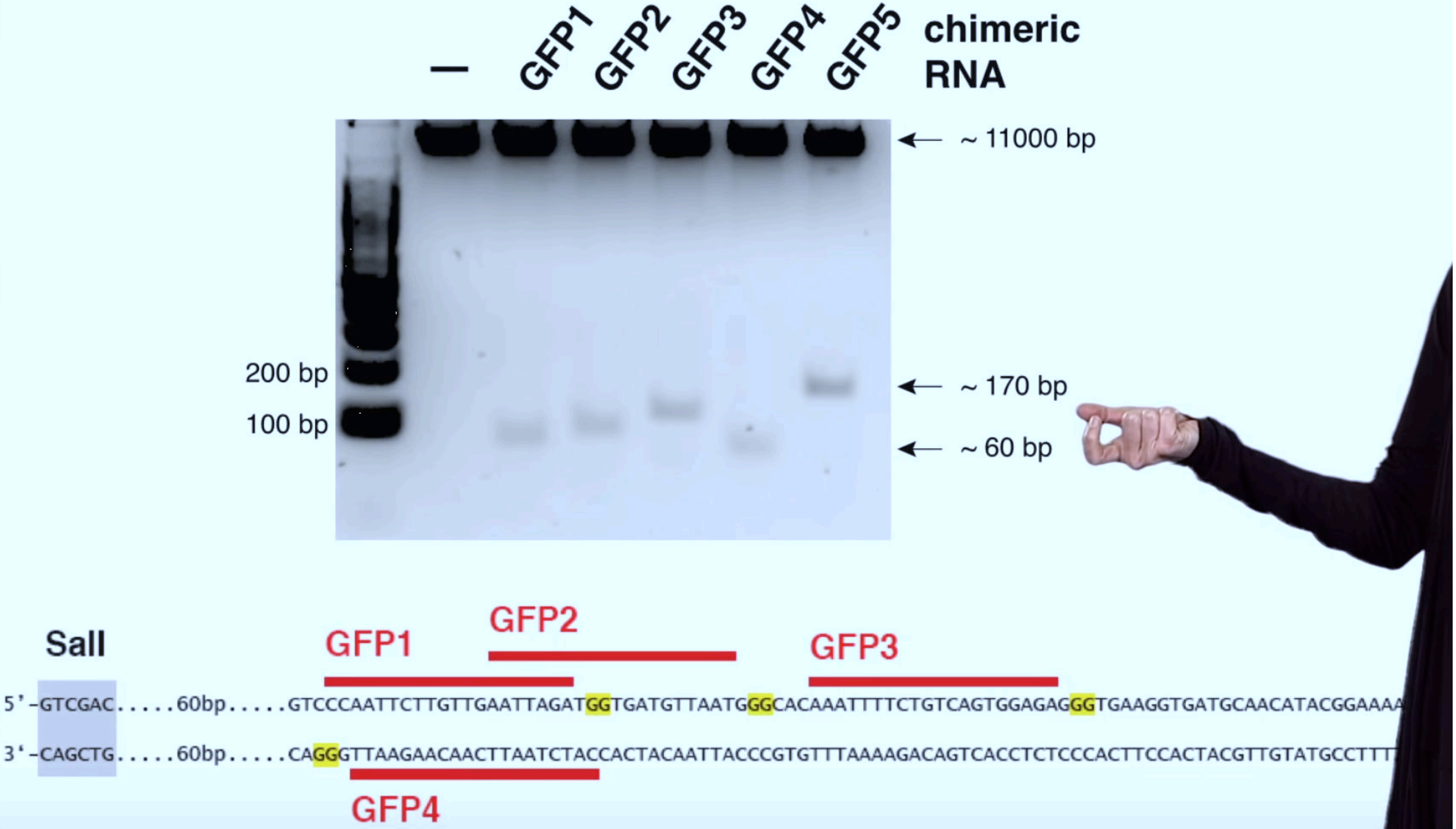
6:29 / 16:41

Jinek *et al. Science* **337**, 816 (2012)



Programmed Cas9 cleaves DNA at specified sites
AmpR 9522...10181
~ 7400 bp
2740 AvrII
~ 3700 bp
GFP 6295...7269
target region 6439...6558
6373 SalI
SalI
GFP1
GFP2
GFP3
5'-GTCGAC.....60bp.....GTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAAC
3'-CAGCTG.....60bp.....CAGGGTTAAGAACAACTTAATCTACCACTACAATTACCCGTGTTTAAAAGACAGTCACCTCTCCCACTTCCACTACGTTGTATGCCTTTT
GFP4
Play (k)
7:08 / 16:41
iBiology.org

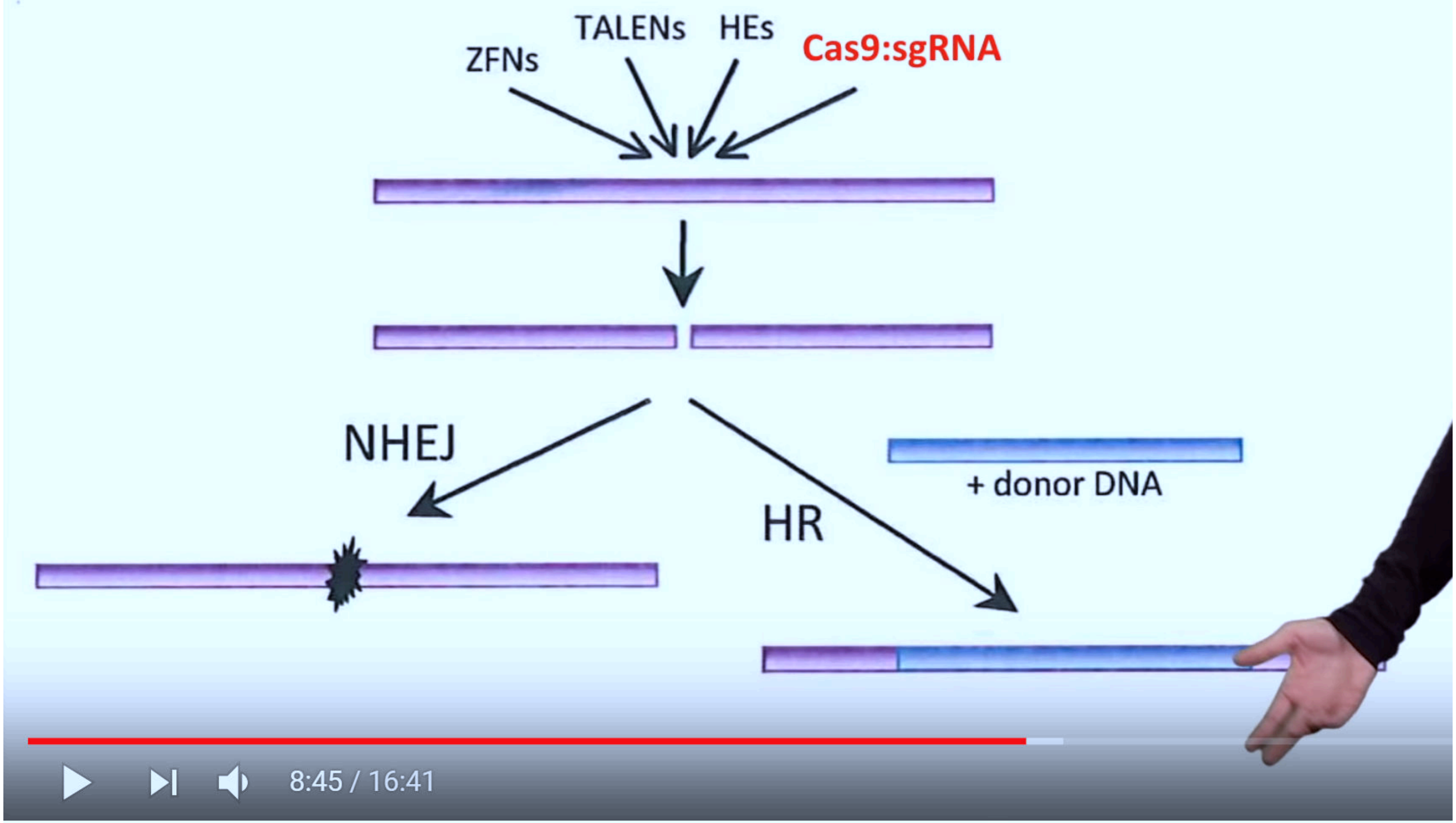
Programmed Cas9 cleaves DNA at specified sites

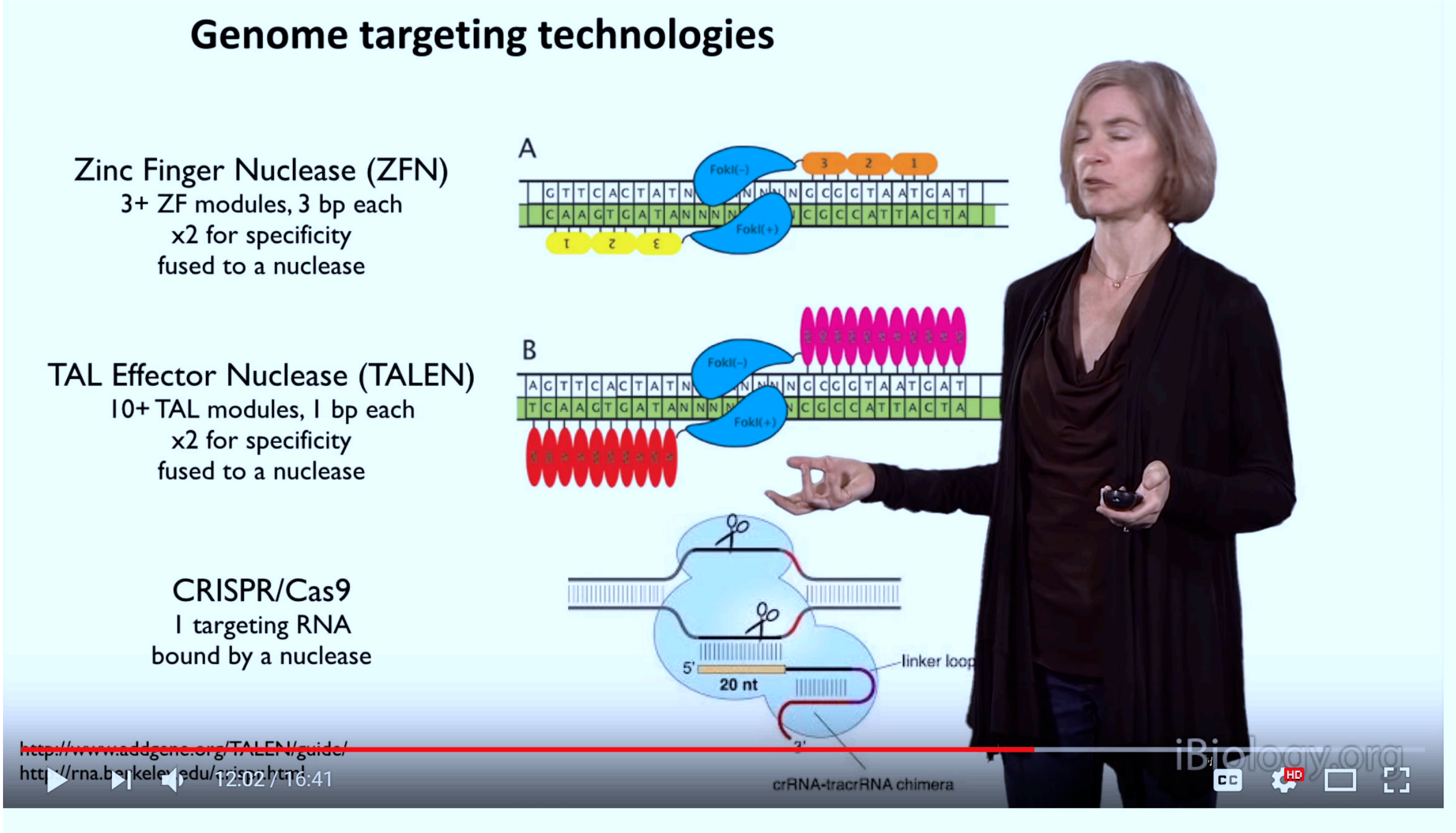


Jinek *et al.* *Science* 337, 816 (2012)

8:01 / 16:41

Genome editing begins with dsDNA cleavage



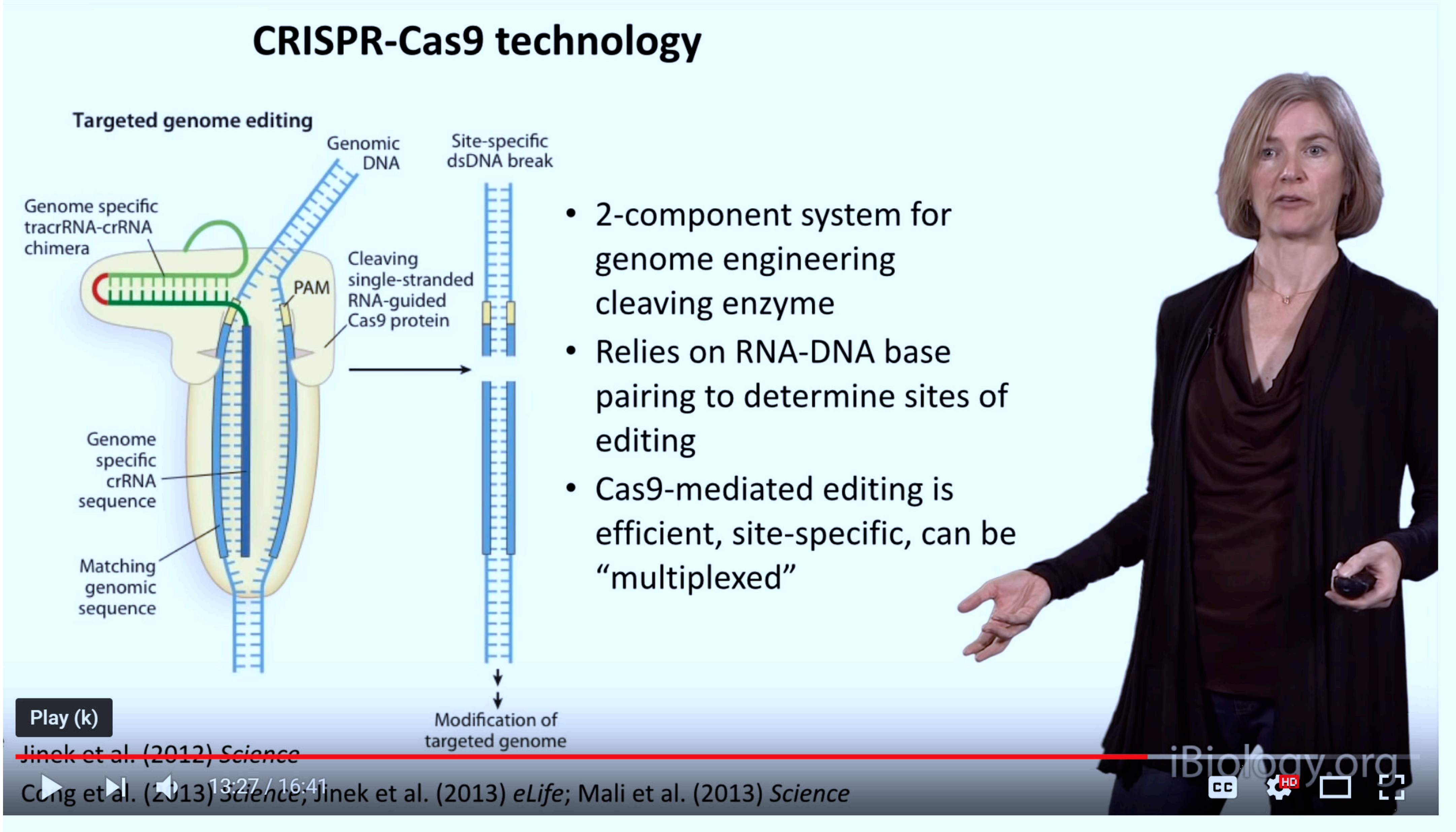


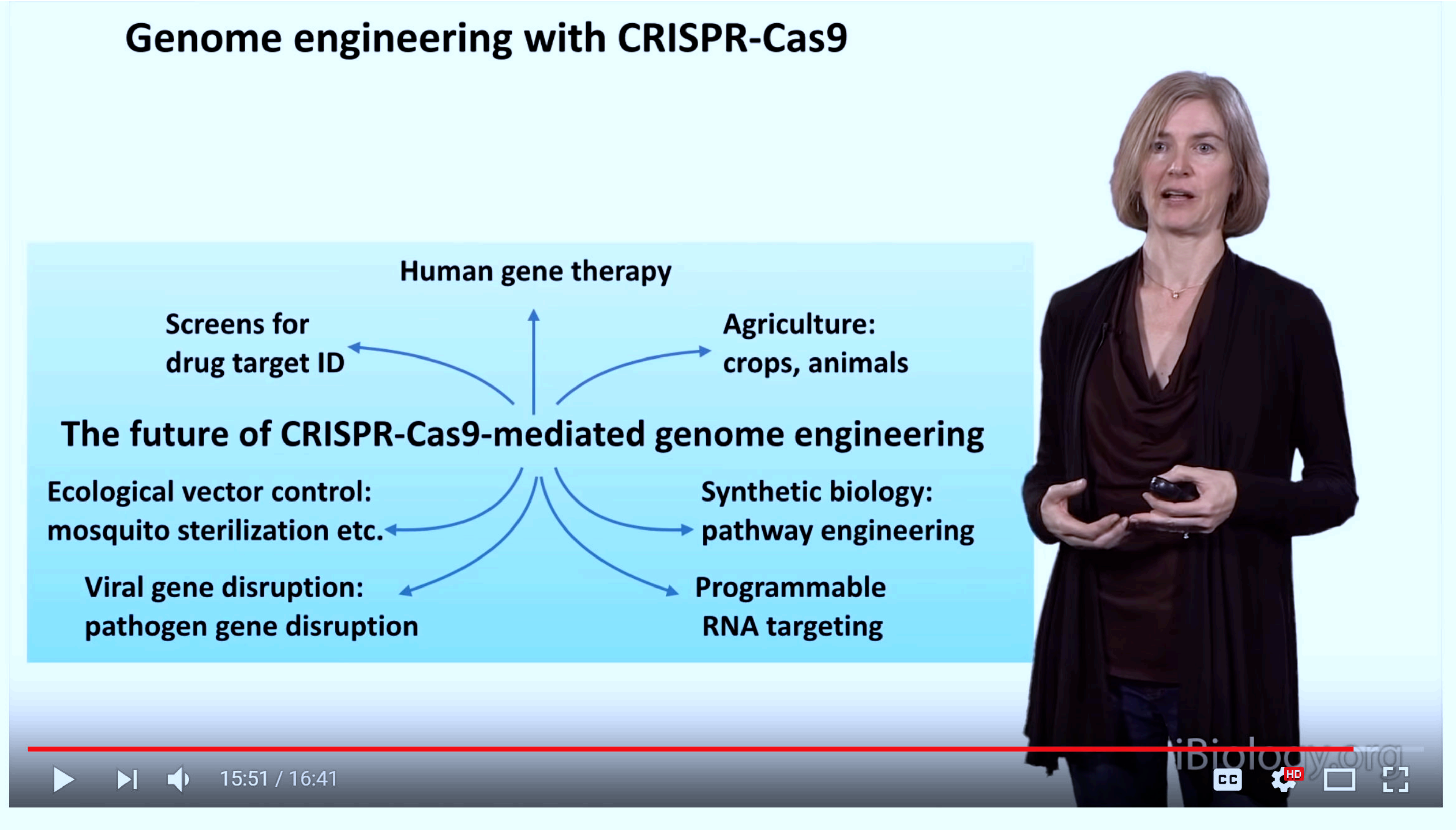
Genome targeting technologies
Zinc Finger Nuclease (ZFN)
3+ ZF modules, 3 bp each
x2 for specificity
fused to a nuclease
TAL Effector Nuclease (TALEN)
10+ TAL modules, 1 bp each
x2 for specificity
fused to a nuclease
CRISPR/Cas9
1 targeting RNA
bound by a nuclease
A
B
FokI(–)
FokI(+)
5'
20 nt
3'
linker loop
crRNA-tracrRNA chimera
http://www.addgene.org/TALEN/guide/
12:02 / 16:41
iBiology.org

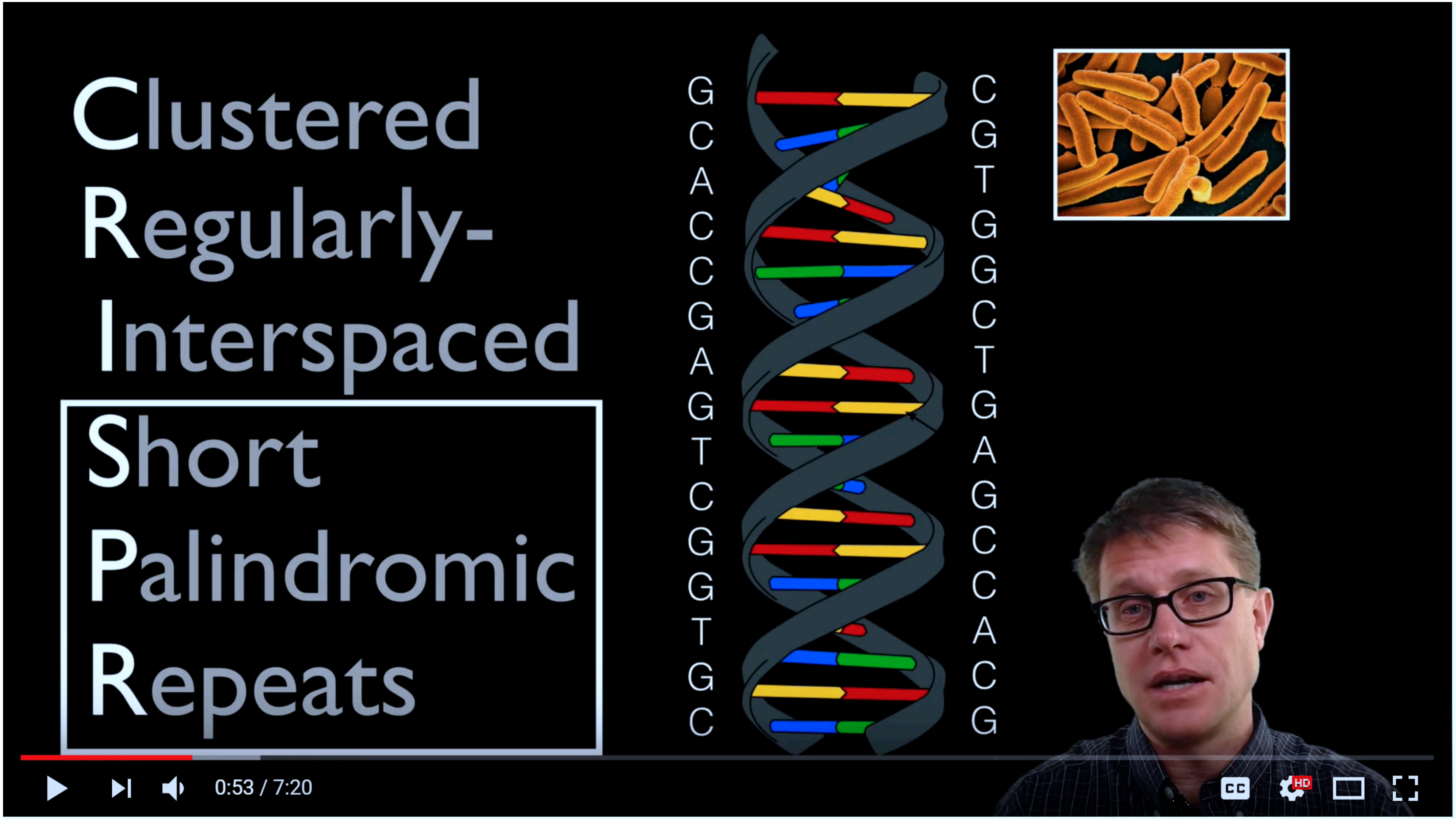
CRISPR-Cas9 technology: Fundamental to Biology's IT Toolbox

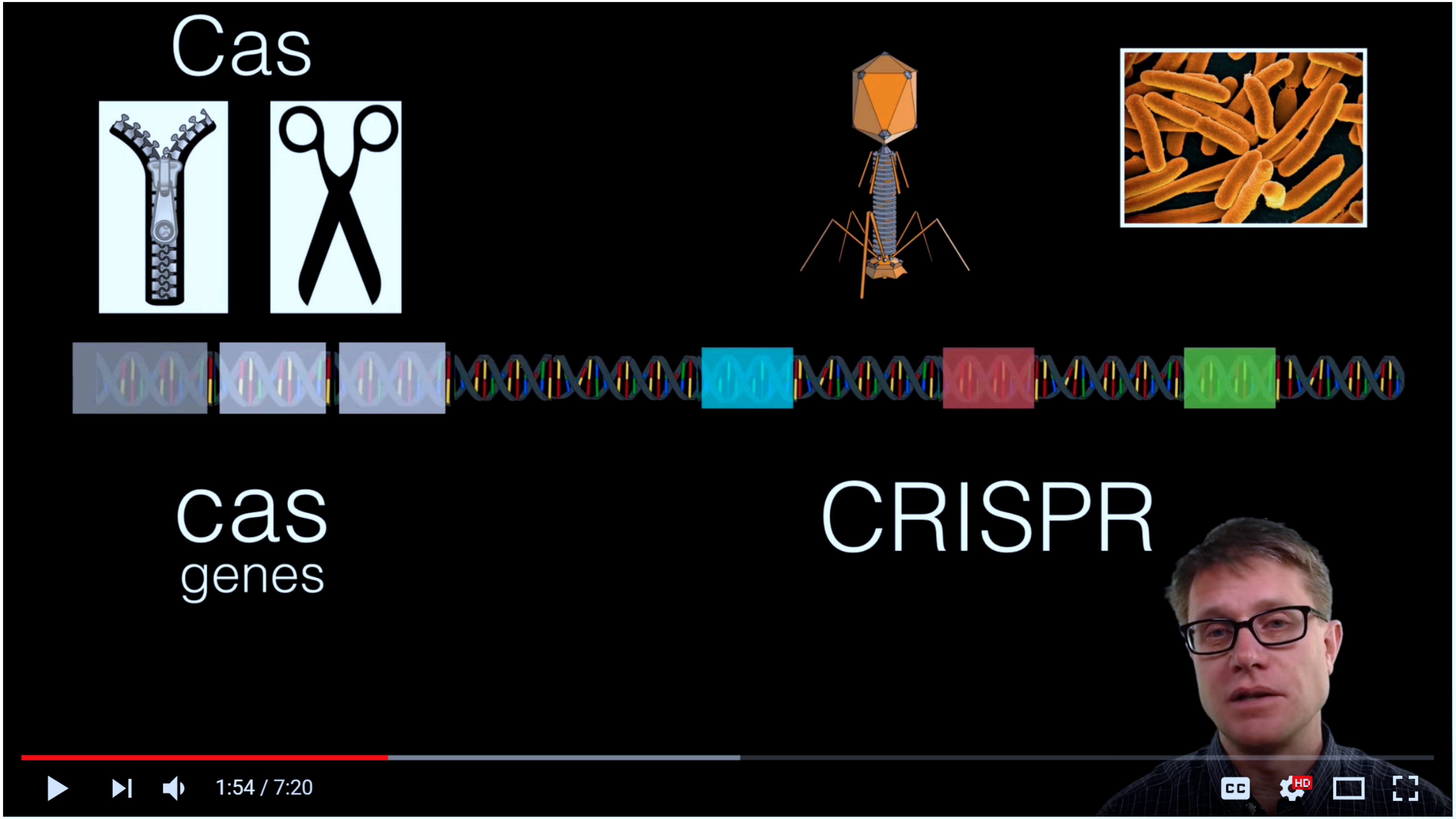
- DNA structure/sequencing
- Restriction enzymes
- PCR
- Genome editing

13:04 / 16:41


CRISPR-Cas9 technology
Targeted genome editing
Genomic DNA
Site-specific dsDNA break
Genome specific tracrRNA-crRNA chimera
PAM
Cleaving single-stranded RNA-guided Cas9 protein
Genome specific crRNA sequence
Matching genomic sequence
Modification of targeted genome
• 2-component system for genome engineering cleaving enzyme
• Relies on RNA-DNA base pairing to determine sites of editing
• Cas9-mediated editing is efficient, site-specific, can be "multiplexed"
Jinek et al. (2012) Science
Cong et al. (2013) Science; Jinek et al. (2013) eLife; Mali et al. (2013) Science
Play (k)
13:27 / 16:41
iBiology.org


Genome engineering with CRISPR-Cas9
Human gene therapy
Screens for
drug target ID
Agriculture:
crops, animals
The future of CRISPR-Cas9-mediated genome engineering
Ecological vector control:
mosquito sterilization etc.
Synthetic biology:
pathway engineering
Viral gene disruption:
pathogen gene disruption
Programmable
RNA targeting
15:51 / 16:41
iBiology.org


Clustered
Regularly-
Interspaced
Short
Palindromic
Repeats
G C A C C G A G T C G G T G C
C G T G G C T G A G C C A C G
0:53 / 7:20
CC
HD



Cas
cas
genes
CRISPR
1:54 / 7:20

Totipotent, Pluripotent & Multipotent What is the Difference!!!

Embryonic Stem Cells

found in embryo

1. Totipotent - whole

total

zygote

2. Pluripotent - several

found in inner cell mass

inner cell mass

blastocyst

Adult Stem Cells

found in many organs

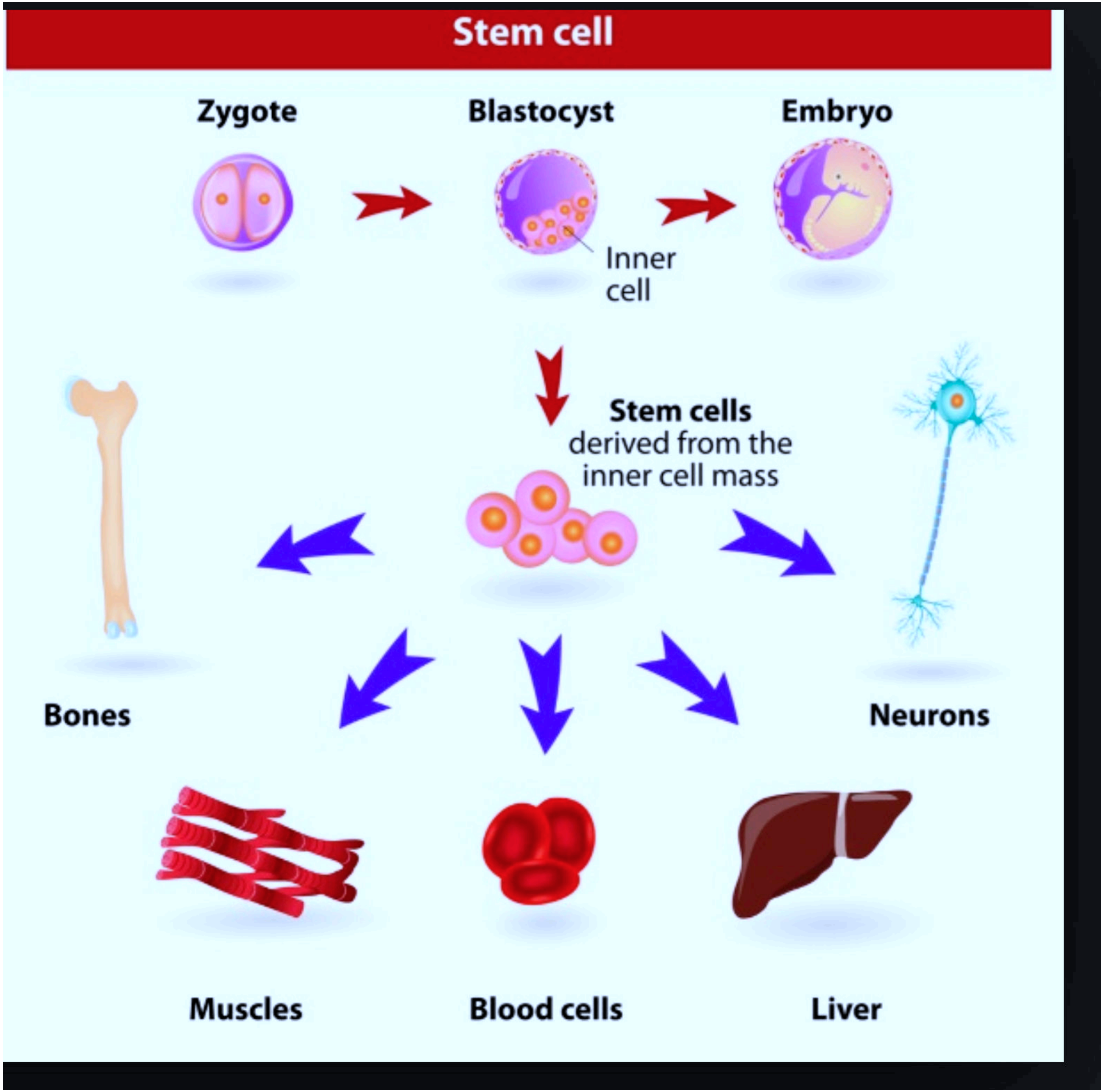
3. Multipotent - a few in this case

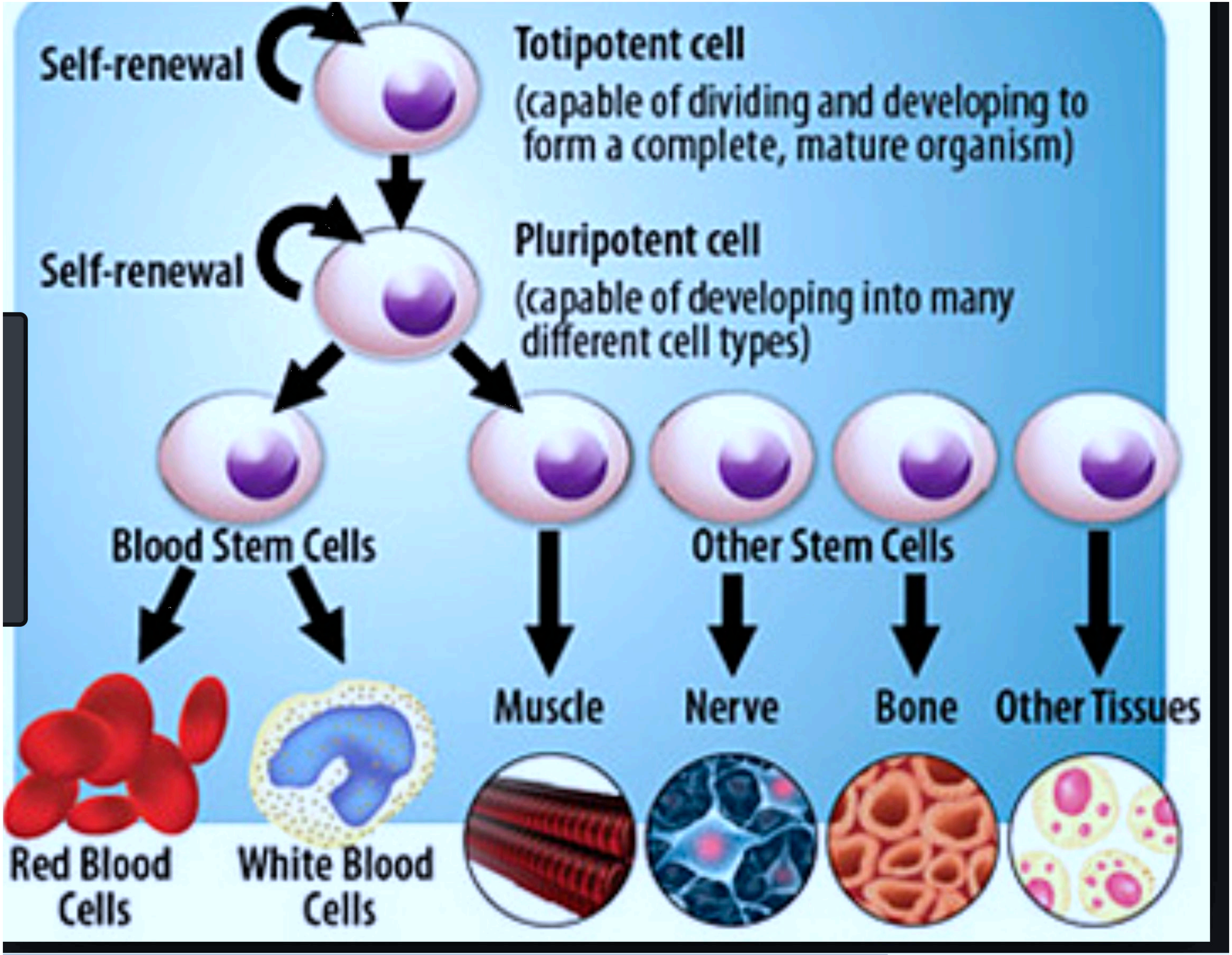
specialization potential is limited to one or more cell lines

e.g. Mesenchymal Stem Cell → Bone

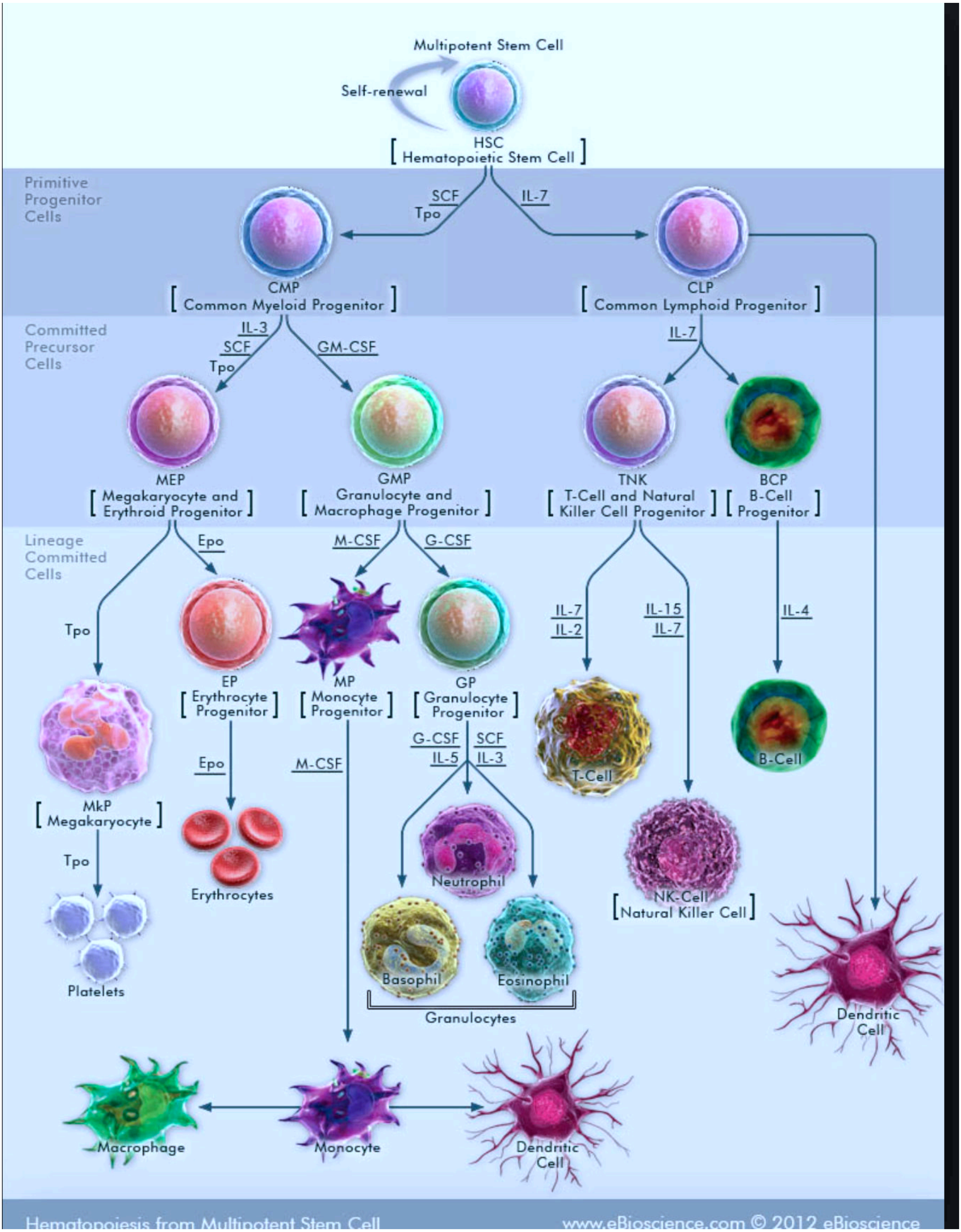
e.g. Mesenchymal Stem Cell → Cartilage

e.g. Mesenchymal Stem Cell → Connective tissue

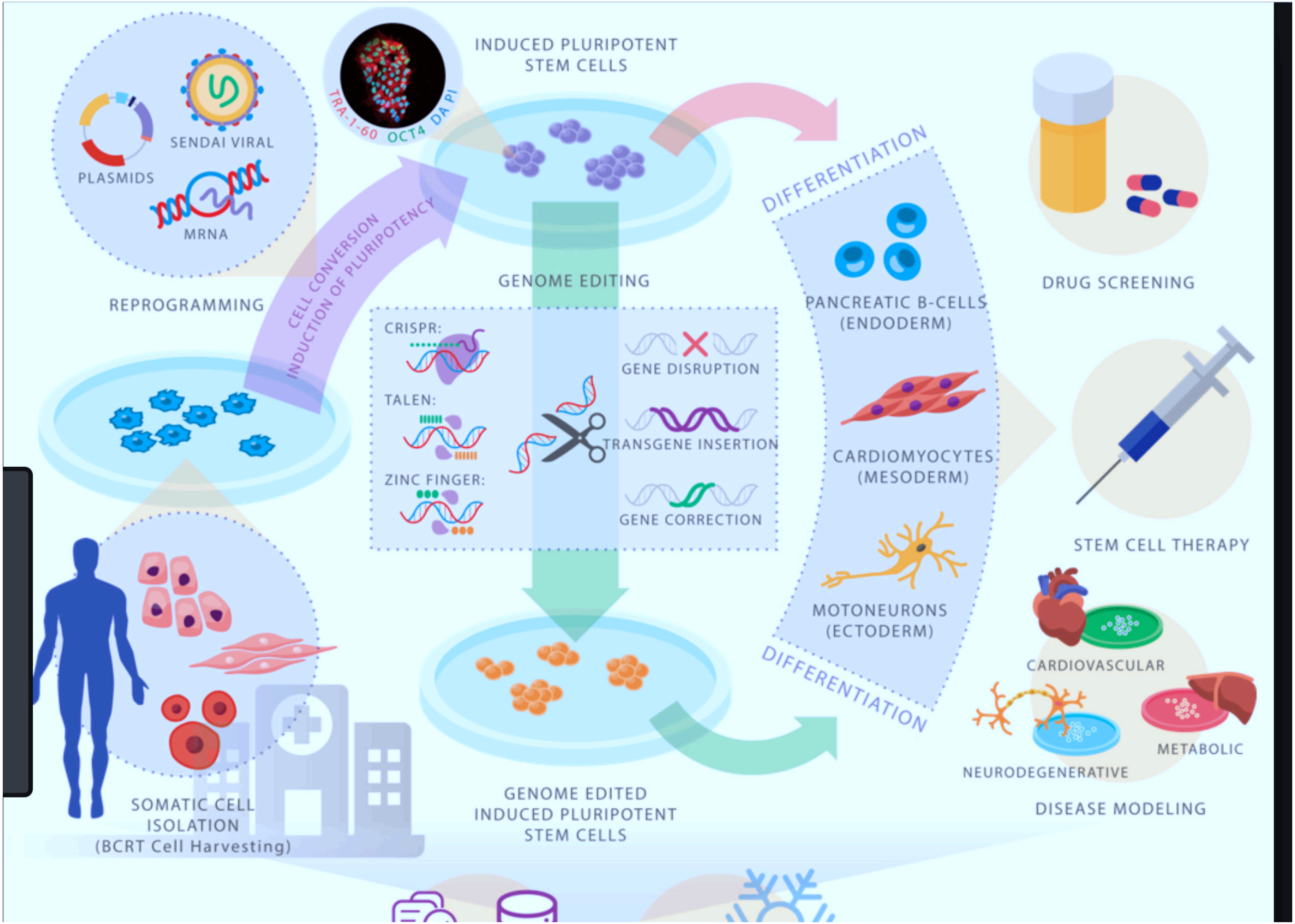

Stem cell
Zygote
Blastocyst
Embryo
Inner
cell
Stem cells
derived from the
inner cell mass
Bones
Neurons
Muscles
Blood cells
Liver



Self-renewal
Totipotent cell
(capable of dividing and developing to form a complete, mature organism)
Self-renewal
Pluripotent cell
(capable of developing into many different cell types)
Blood Stem Cells
Other Stem Cells
Muscle
Nerve
Bone
Other Tissues
Red Blood Cells
White Blood Cells



Multipotent Stem Cell
Self-renewal
HSC
Hematopoietic Stem Cell
Primitive Progenitor Cells
SCF
Tpo
IL-7
CMP
Common Myeloid Progenitor
CLP
Common Lymphoid Progenitor
Committed Precursor Cells
IL-3
SCF
Tpo
GM-CSF
IL-7
MEP
Megakaryocyte and Erythroid Progenitor
GMP
Granulocyte and Macrophage Progenitor
TNK
T-Cell and Natural Killer Cell Progenitor
BCP
B-Cell Progenitor
Lineage Committed Cells
Epo
M-CSF
G-CSF
Tpo
IL-7
IL-2
IL-15
IL-7
IL-4
EP
Erythrocyte Progenitor
MP
Monocyte Progenitor
GP
Granulocyte Progenitor
T-Cell
B-Cell
G-CSF
IL-5
SCF
IL-3
Epo
M-CSF
MkP
Megakaryocyte
Neutrophil
NK-Cell
Natural Killer Cell
Tpo
Erythrocytes
Basophil
Eosinophil
Granulocytes
Platelets
Dendritic Cell
Macrophage
Monocyte
Dendritic Cell
Hematopoiesis from Multipotent Stem Cell
www.eBioscience.com © 2012 eBioscience



INDUCED PLURIPOTENT STEM CELLS
TRA-1-60 OCT4 DAPI
PLASMIDS
SENDAI VIRAL
MRNA
REPROGRAMMING
CELL CONVERSION
INDUCTION OF PLURIPOTENCY
GENOME EDITING
CRISPR:
TALEN:
ZINC FINGER:
GENE DISRUPTION
TRANSGENE INSERTION
GENE CORRECTION
DIFFERENTIATION
PANCREATIC B-CELLS (ENDODERM)
CARDIOMYOCYTES (MESODERM)
MOTONEURONS (ECTODERM)
DIFFERENTIATION
DRUG SCREENING
STEM CELL THERAPY
CARDIOVASCULAR
NEURODEGENERATIVE
METABOLIC
DISEASE MODELING
SOMATIC CELL ISOLATION
(BCRT Cell Harvesting)
GENOME EDITED INDUCED PLURIPOTENT STEM CELLS